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ENZYMS OF MILK AND BUTTER[1]

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INTRODUCTION

The deterioration of butter during storage is often attributed to the enzyms in the buttermilk which it contains. There has been some experimental study of the matter; but most of the published results of these investigations yield little information concerning the actual enzym content of butter, because of the fact that the studies dealt chiefly with the chemical changes in the butter itself during storage, and sufficient care was not used to prevent possible contamination by microorganisms. In fact, a survey of the literature dealing with the enzyms of milk and its products shows a lamentable lack of clearness on the part of the investigators in distinguishing between the enzyms of milk itself and those which are due to bacterial infections.

Rogers (*16*)[2] found lipase to be the cause of the increase in the acidity of canned butter on standing. Rahn, Brown, and Smith (*15*) stated that

> all (storage) butter investigated showed an increase of "amid nitrogen," i. e., nitrogen not precipitated by copper sulphate, tannic acid, or phosphotungstic acid.

Their methods were shown to be at fault by Rogers, Berg, Potteiger, and Davis (*17*), who could observe

> no evidence of an increase in soluble nitrogen in butter on long standing at 0° F., even when the conditions of manufacture were most favorable to such changes.

They could detect no proteolysis in buttermilk held a long period of time in cold storage to which 18 per cent of sodium chlorid had been added, but active bacterial proteases, pepsin, and trypsin were not completely inhibited in their action by these adverse conditions. Lactose, they concluded, was oxidized only when a trace of iron and peroxid were added.

During the summer of 1915 one of us (Dahlberg), as a part of his duties in the Division of Dairy and Animal Husbandry of the Minnesota Agricultural Experiment Station, prepared several lots of butter under carefully controlled conditions of manufacture and placed them in stor-

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[2] Reference is made by number (italic) to "Literature cited," pp. 448-450.

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age, in order to study the effect of varying methods of manufacture and storage upon the keeping qualities of the butter. From time to time studies of the bacterial development in the stored butter were made. The results of this work have recently been published elsewhere (*25*).

Since the conditions of manufacture and the bacterial development in these butters were known, they afford excellent material for a study of the enzym content of the butter after storage, and such a study was accordingly undertaken.

REVIEW OF PREVIOUS WORK

The following types of enzyms have been reported to be normally present in cow's milk: an amylase (*27*), a lipase (*9*), proteases (*1–5, 7, 22*) a peroxidase (*19*), salolase (*22*), catalase (*12, 18, 20*), reductase (*20*), and a lactose-fermenting enzym (*21*). Vandevelde (*22*), however, concludes that no lipase can be found in fresh milk and that salolase is present in only very slight amounts; while Grimmer (*10*), as a result of his study of the enzyms of the mammary glands, concludes that salolase, peroxidase, and catalase are the only enzyms which are normally secreted with milk. Further, Van de Velde and Landsheere (*23*) found no amylase in milk and criticize Spolverini's observations concerning peroxidase in milk, holding that its presence there is due to bacterial contamination.

These reports indicate something of the confusion which exists with reference to the normal enzyms of milk. The contradictory evidence which has been presented undoubtedly results, in part at least, from the rather crude methods of study of enzym action which were in use at the time when these investigations were in progress. Unfortunately, little attention has been given to these matters during recent years, when more refined methods of study might have given more concordant results.

The question as to whether any enzyms which may be present in milk will be carried down with the butter in churning has not been generally discussed. Kooper (*13*) states that during butter making catalase remains behind in the buttermilk and is therefore not a direct constituent of butter fat. It might be assumed, however, that any buttermilk which remains in the butter would contain the same enzyms that were present in the original milk. In fact, Hesse (*12*), who found small amounts of catalase in butter, calculated his results on the basis of its buttermilk content. Furthermore, since butter fat is undoubtedly an emulsion colloid, it might be assumed to carry with it into the butter, in the form of absorption complexes, large proportions of the enzyms originally present in the milk. But no definite information concerning these matters, other than that cited in the opening paragraphs of this paper, has been published.

EXPERIMENTAL WORK

I. INVESTIGATIONS CONCERNING GALACTASE

The normal proteolytic enzym of milk, which aids in the slow decomposition of milk proteins into peptones, amino acids, and ammonia, was given the name "galactase" by Babcock and Russell (*1–5*). This name is unfortunately not in accord with the present system of nomenclature, and the enzym ought properly to be called "casease" or "lactalbuminase"; but, since the name suggested by Babcock and Russell has come into common use and its significance is properly understood, we decided to continue its use.

Before undertaking the proposed studies of the galactase content of milk, cream, skim milk, buttermilk, and butter it was necessary to determine upon certain details of technic which had not been satisfactorily worked out by former investigators. This preliminary work may be briefly summarized as follows:

(*a*) LENGTH OF PERIOD OF ACTION.—Previous experimental data give quantitative measures of proteolytic activity for periods varying from 1 hour for certain pepsin studies (*8*) up to the practically prohibitive time of nearly 3 years for galactase (*14*). The time in which most investigators have allowed galactase to act, in order to obtain comparative results, has varied from 30 days to 1 year. This appears to be unnecessarily long. The analytical data presented by Babcock and Russell give an average increase in the soluble-nitrogen content of a number of samples of skim milk, when expressed as percentage of the total nitrogen, of 13.49 per cent for the first 8 days and only 5.66 per cent for the following 12 days. One sample of skim milk increased 26 per cent in soluble nitrogen during the first 7 days, 2 per cent the following 7 days, and to increase the soluble nitrogen content another 26 per cent required a period of 3 days. There is very little difference in the comparative galactase content of various skim milks when analyses made at the end of 7 days or of 6 months are compared. In the present work the period of action was limited to 4 days, since it was found that the soluble nitrogen in skim milk increased 10 or 12 per cent during this time.

(*b*) MEASUREMENT OF HYDROLYSIS BY MEANS OF THE NINHYDRIN REACTION.—The ninhydrin method, as proposed by Harding and MacLean (*11*) for the determination of amino-acid or nitrogen, was compared with the official method of the Association of Official Agricultural Chemists (*26*) for determining the increase in soluble nitrogen in different samples of milk due to protease action (Table I). In the ninhydrin determination the sample was diluted 1 to 10, and 1 c. c. of this used in each analysis, but the results were calculated to the 10 c. c. basis. Bacterial action was inhibited by 0.5 per cent of chloroform. All samples in all the work reported in this paper were incubated at 40° C. for four days.

TABLE I.—*Results with ninhydrin method of estimating the rate of hydrolysis of the proteins of milk*

Sample.	Total nitrogen.	Initial soluble nitrogen.	Initial ninhydrin nitrogen.	48-hour soluble nitrogen.	48-hour ninhydrin nitrogen.	Increase in soluble nitrogen.	Increase in ninhydrin nitrogen.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Boiled skim milk	0.0586	0.0033	0.0029	0.0039	0.0032	1.02	0.51
Skim milk + 3 per cent of sodium chlorid	.0586	.0108	.0029	.0137	.0031	4.95	.34
Skim milk	.0586	.0108	.0029	.0177	.0032	11.77	.51

The ninhydrin method indicated no increase in nitrogen when there was an actual increase of 11.5 per cent in the soluble nitrogen. This was further substantiated by work on a pure casein solution. This gave only a trace of nitrogen by the ninhydrin method, with no increase after evident proteolysis had taken place. This method of estimating the rate of hydrolysis of proteins was therefore abandoned as not being applicable to investigations with milk proteins.

(*c*) THE INFLUENCE OF CHLOROFORM ON PROTEOLYSIS.—To prevent bacterial action in the samples, chloroform was added in amounts found ample by Harding and MacLean (*11*) and Van Slyke (*24*). Skim milk to be analyzed at varying intervals extending over a long period of time was treated with 1 per cent chloroform, which, according to the above investigators and Babcock and Russell (*5*), should have only slightly inhibited proteolysis. As shown in Table II, the soluble nitrogen of this skim milk did not increase on standing. In verifying this result the soluble nitrogen was obtained by a Kjeldahl determination of the nitrogen in the total filtrate from the casein precipitation. Closer agreement in the duplicates could be obtained from the filtrates than from the casein itself.

TABLE II.—*Influence of chloroform on the proteolysis of milk as indicated by soluble nitrogen*

Sample.	Chloroform.	Total nitrogen.	Initial soluble nitrogen.	Soluble nitrogen after 4 days.	Increase in soluble nitrogen as per cent of total.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	
1. Skim milk	0.5	0.0583	0.0129	0.0183	9.26
	1.0	.0583	.0130	.0114	−2.74
2. Skim milk	.5	.0531	.0115	.0170	10.35
	1.0	.0531	.0115	.0120	.94
3. Skim milk	.5	.0406	.0119	.0136	4.18
	1.0	.0406	.0119	.0103	−3.94
4. Skim milk	1.0	.0547	.0106	.0079	−4.93
5. Buttermilk, neutral	.5	.0551	.0125	.0201	13.60
	1.0	.0551	.0125	.0174	9.80
6. Buttermilk, neutral	1.0	.0463	.0064	.0059	−1.08

Sample 5 was buttermilk obtained from a small hand churning and probably contained 1 per cent of butter fat. Since fat destroys the anesthetic property of chloroform by combining with it, the results of No. 5 should not be considered. In every other case 1 per cent of chloroform in skim milk or buttermilk completely inhibited proteolysis.

(*d*) THE INFLUENCE OF SODIUM CHLORID ON PROTEOLYSIS.—The possibility that the sodium chlorid present in ordinary butter might inhibit proteolytic activity led us to make a study of the effect of varying percentages of sodium chlorid added to skim milk upon the rate of production of soluble nitrogen by galactase. After the period of incubation the unchanged casein was precipitated from the milk by means of dilute acetic acid and the soluble nitrogen in the filtrate determined by the Kjeldahl method. The samples were preserved during the incubation by 0.75 per cent chloroform. The following results were obtained (Table III).

TABLE III.—*Influence of sodium chlorid on the proteolysis of milk (with 0.75 per cent of chloroform)*

Sodium chlorid added.	Total nitrogen.	Initial soluble nitrogen.	Soluble nitrogen after 21 days.	Soluble nitrogen after 72 days.	Increase.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0.0 boiled	0.0561	0.0055	0.0051	0.0034	−3.74
.0	.0561	.0121	.0188	.0198	13.72
.5	.0561	.0121	.....	.0171	8.91
1.0	.0561	.0121	.0142	.0131	1.78
15.0	.0534	.0121	.....	.0110	−2.06
20.0	.0528	.0121	.0120	.0103	−3.40

The effect of sodium chlorid is marked; both 15 and 20 per cent, the concentration of the salt brine in butter, stopped all proteolysis. The very slight increase in the soluble nitrogen content of the skim milk containing 1 per cent of the salt is partially due to the excessive chloroform used, as the following tests, in which only 0.5 per cent of the anesthetic was used, will show (Table IV).

TABLE IV.—*Influence of sodium chlorid on the proteolysis of milk (with 0.5 per cent of chloroform)*

Sodium chlorid added.	Total nitrogen.	Initial soluble nitrogen.	Soluble nitrogen after 5 days.	Increase.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0.0 boiled	0.0586	0.0033	0.0034	0.51
.0	.0586	.0108	.0242	22.86
.8	.0586	.0108	.0201	15.87
3.0	.0586	.0108	.0143	5.97

The actual checking of the increase in soluble nitrogen due to the addition of 1 per cent of sodium chlorid was approximately 10 per cent in either case, but 3 per cent of the salt did not entirely prevent proteolysis.

(*e*) Occurrence of galactase in milk during process of butter making.—In following the protease of the fresh milk to the finished butter, the reaction of the various products was in every case brought to that of the fresh milk—that is, 10 c. c. were exactly neutralized by 1.5 c. c. of *N*/10 alkali when phenolphthalein was used as an indicator, by dipping a stick of sodium hydroxid into the sample. If the sample became too alkaline, it was neutralized by some of the original sample so that dilution was avoided. The various samples were treated with chloroform in the following proportions: The skim milk, buttermilk, and bowl contents, 0.5 per cent; the milk, 2.5 per cent; and the cream, which contained 23 per cent of butter fat, 5 per cent. The "bowl contents" was an emulsion of the slime in the wash water held in the bowl. An equal volume of boiled skim milk was added as a substrate, but the increase in the soluble nitrogen was calculated on the basis of the bowl contents alone. The "cream during ripening" refers to the proteolysis occurring during the 10 hours it was ripened at 85° F. and the 20 hours it was held at 54° previous to churning.

Table V.—*Presence and concentration of galactase in milk and butter as indicated by the increase of soluble nitrogen*

Sample.	Total nitrogen.	Initial soluble nitrogen.	Soluble nitrogen after 4 days.	Increase.	Initial percentage of total nitrogen as casein.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	
Skim milk	0. 0583	0. 0129	0. 0183	11. 10	77. 87
Whole milk	. 0544	. 0113	. 0198	15. 62	79. 23
Cream	. 0403	. 0076	. 0142	16. 37	81. 14
Bowl contents	. 0189	. 0046	. 0262	114. 28	89. 93
Cream during ripening	. 0403	. 0076	. 0081	1. 66	.....
Cream after ripening	. 0403	. 0081	. 0139	14. 39	.....
Buttermilk	. 0551	. 0125	. 0201	13. 60	.....

It appears from these data that in separating milk, galactase is taken out of the skim milk part, slightly increased in the cream, and highly concentrated in the separator slime. While no relationship exists between the increase in soluble nitrogen and the total nitrogen, it is evident that the factors at work during milk separation which increase the percentage of casein in the total nitrogen also increase the galactase content. The cream underwent a slight proteolytic digestion during the ripening process, but the proteolysis after souring and neutralization was less than that of the sweet cream. This indicates that the chief

proteolytic enzym of milk is not of bacterial origin, as Olson (*14*) recently reported.

(*f*) Amount of galactase contained in butter.—The butter used in the experiments represented both good and bad qualities. The "fresh dairy" butter was made from the cream obtained from the milk of the University Farm herd, and was soured spontaneously without pasteurization. The "fresh creamery" butter was made in a cooperative creamery from sweet pasteurized cream ripened by a commercial starter. Both of these butters were of extra-fine quality. The "stored dairy" butter was the same as the "fresh dairy," except that it had been held in cold storage for eight months. The "fresh centralized" butter was made from the sour cream just as it was received by a central creamery. The last two butters mentioned were of poor quality. The "stored centralized" butter was made in the same way as the "fresh centralized," but had been held eight months in cold storage. It was of extremely poor quality.

No effort was made to obtain a pure enzym extract from the butter. A known weight, usually 400 gm., of butter was melted at 45° C. in two long glass tubes about an inch in diameter. After the separation was complete the clear fat was hardened by immersing the tubes in cold water, and the curd solution was then washed out. If an excess of fat remained in the extract, it was removed by rewarming and centrifuging. This extract was then dialyzed at a temperature never exceeding 13°C., in a parchment dialyzer, until free of sodium chlorid, the last six or eight hours of dialysis being with distilled water. This curd was made up to a given volume and used at once in the various tests.

The acetone-ether method of obtaining a fat-free, dry powder was also tried, but so high a percentage of sodium chlorid was left in the powder that dialysis was necessary. Consequently this method had no advantages over the other, and the enzyms probably would have been weakened by the precipitation.

The casein was precipitated by the optional official method (*26*, p. 118), rather than the official method because filtering was often more rapid, the volume to be filtered much less, and a clear filtrate more easily obtained. (Thirteen analyses of chloroformed-skim milk and butter-curd extracts gave an average of 0.00104 gm. more nitrogen in the form of casein by the use of alum as the precipitant; hence, the methods should not be used interchangeably.) A clear filtrate was more easily obtained and an excess of substrate assured by the addition of boiled skim milk to the curd solutions. In a few cases the filtering had to be done on a "Büchner funnel" through three filter papers and repeated 10 to 20 times to obtain a perfectly clear filtrate. Bacterial action was prevented by 1 per cent chloroform instead of 0.5 per cent because the extracts contained considerable fat (Table VI).

TABLE VI.—*Presence of galactase in butter, as indicated by the increase in soluble nitrogen*

Kind of butter.	Concentration.		Initial soluble nitrogen.	Soluble nitrogen after 4 days.	Increase.	Increase for 10 gm. of butter.
	Ratio, butter to curd extract.	Ratio, curd extract to boiled skim milk added.				
			Gm.	*Gm.*	*Gm.*	*Gm.*
1. Fresh dairy	2:1	50:100	0.0098	0.0108	0.0010	0.0015
Fresh dairy, boiled	2:1	50:100	.0091	.0091	.0000	.0000
2. Fresh dairy	2:1	50:100	.0080	.0090	.0000	.0015
Fresh dairy, boiled	2:1	50:100	.0070	.0045	.0025	.0000
3. Stored dairy	3:2	50:50	.0043	.0063	.0020	.0027
4. Stored centralized	2:1	50:50	.0042	.0078	.0036	.0036
Stored centralized boiled	2:1	50:50	.0039	.0036	.0003	.0000
5. Fresh creamery	3:2	50:50	.0049	.0044	.0005	.0000

The two samples of fresh dairy butter gave uniform and measurable proteolytic action, while one sample of fresh pasteurized creamery butter showed no digestion of the casein. The increase in the soluble nitrogen of the stored butter was nearly twice that of the fresh butter.

II. LIPASE CONTENT OF BUTTER

Lipase hydrolyzes fats into alcohols and fatty acids, causing an increase in the acidity of the media acted upon. The increase in the acidity of butter on standing is supposed to be due to this enzym (*16*).

The curd solutions used in testing for lipase activity were just half the volume of the butter. The substrate was either 5 per cent of butter fat or olive oil, and the preservative was 2.5 per cent of chloroform. Ten c. c. aliquots of the extracts in 10 c. c. of neutral 95 per cent alcohol were titrated against *N/40* sodium hydroxid, with phenolphthalein as indicator.

TABLE VII.—*Presence of lipase in butter as shown by the increase of acidity*

Kind of butter.	Initial acidity.	Acidity after 4 days.	Increase over boiled.
	Per cent.	*Per cent.*	*Per cent.*
1. Fresh dairy, boiled	0.2	1.3	0.0
Fresh dairy	.2	1.1	.0
2. Fresh unsalted dairy, boiled	5.3	5.3	.0
Fresh unsalted dairy	6.3	6.6	.3
3. Stored unsalted dairy	5.5	5.5	.0
4. Fresh centralized	2.7	3.0	.3
5. Stored centralized	2.4	3.5	1.1
6. Fresh creamery	1.8	2.2	.4

The extracts from the unsalted butters were not dialyzed. This accounts for their higher initial acidity.

Lipase activity during four days at 40° C. was too small to be accurately measured, except in the extract from the stored centralized butter.

III. OXIDASE CONTENT OF MILK AND BUTTER

Since the investigations of oxidase activity in milk have been limited to a few easily oxidized substances and since oxidases show a specificity toward chemicals in their action, it was thought desirable to investigate by Bunzel's method the action of milk on several of the common chromogens (7). Four c. c. of milk were used in each test. Negative results were obtained with paraphenylene diamine, phenolphthalein, hydroquinon, pyrocatechin, phloroglucin, α-naphthol, and para-, meta-, and ortho-cresols. Positive results were obtained with metol and pyrogallol; but since the action of the latter was about 20 per cent the greater, pyrogallol alone was used for the final determinations. The results given in Table VIII were obtained in duplicate, the duplicates agreeing very closely.

TABLE VIII.—*Estimation of oxidase in milk as shown by the oxygen absorption of pyrogallol*

[Readings are expressed as millimeters of mercury, each millimeter representing an absorption of 0.025 c. c. of oxygen.]

Time.	Skim milk 1.	Whole milk.		Skim milk 2.	
		Raw.	Boiled.	Raw.	Boiled.
Minutes.					
60	.....	0. 16	0. 00	0. 00	0. 00
80	.....	. 40	. 00	. 00	. 00
100	.....	. 56	. 00	. 17	. 00
120	0. 95	. 70	. 16	. 17	. 00
140	1. 07	. 85	. 20	. 35	. 00
160	1. 28	. 97	. 27	. 40	. 00
180	1. 33	1. 13	. 40	.....	.....
300	.....	1. 70	. 91	.....	.....
360	.....	1. 97	1. 22	.....	.....
420	.....	2. 25	1. 48	.....	.....
460	.....	2. 32	1. 55	.....	.....

There was no agreement in the results obtained from the skim milk from the same herd on different days; boiling failed to completely inhibit the activity of the whole milk, and the action was very slow in starting. It can not be caused by a normal milk oxidase. In every case a browning of the samples was quite marked before oxygen absorption started.

Seven curd solutions from different butters gave no action with pyrogallol.

IV. CATALASE CONTENT OF MILK AND BUTTER

Storch's method of measuring the catalase of milk, as given by Barthel (*6*), gave very closely agreeing duplicate results and was used in this work (Table IX). In milk the liberation of oxygen ceased in four hours. The curd extracts were equal in volume to the original butter. Five per cent of commercial hydrogen peroxid was added to the samples to be tested, and the concentration of peroxid in the boiled samples at the end of four hours were used as the basis for calculation.

TABLE IX.—*Catalase content of milk and butter as shown by reaction with hydrogen peroxid*

Sample.	Hydrogen peroxid content after 4 hours.		Reduction of hydrogen peroxid in unboiled milk or butter.
	Boiled.	Unboiled.	
	Gm.	*Gm.*	*Gm.*
1. Boiled milk	0.0054	.....	.....
Milk	.....	0.0039	0.0015
2. Boiled fresh dairy butter	.0054	.....	.....
Fresh dairy butter	.....	.0049	.0005
3. Stored dairy butter	.....	.0049	.0005
4. Fresh centralized butter	.....	.0026	.0028
5. Stored centralized butter	.....	.0039	.0015
6. Fresh pasteurized creamery butter	.....	.0048	.0006

The fresh centralized butter did not contain as much catalase as the results show, because a clear filtrate was not obtained. Every sample of butter contained catalase. That the centralized butter gave higher results than the other butter and as high as milk itself agrees with the belief that there is some relationship existing between catalase activity in milk and bacterial growth. Storage did not diminish its activity.

V. PEROXIDASE CONTENT OF MILK AND BUTTER

The common Storch test for heated milk was used on the butter extracts with the following results:

TABLE X.—*Color changes showing the peroxidase content of milk and butter*

Sample.	Time of heating (in minutes).				
	0	5	10	20	30
1. Fresh dairy milk boiled.	White...	White...	White.....	White.....	White.
Fresh dairy milk	...do....	Gray...	Gray.....	Gray.....	Gray.
2. Stored dairy milk	...do....	White...	White.....	Faint gray.	Faint gray.
3. Fresh centralized	...do....	Gray...	Gray.....	Gray.....	Gray.
4. Stored centralized	...do....	White...	Faint gray.	Faint gray.	Faint gray.
5. Fresh pasteurized creamery.	...do....	...do....	White.....	...do.....	Do.

All the samples gave some color change, but for the storage butters and the pasteurized creamery butter it was very slight. Milk diluted 1 to 160 with distilled water and to which 16 per cent of boiled milk was added gave a gray color similar to that of the fresh dairy butter; hence the peroxidase content of butter is very small.

COMPARISON OF MILK AND BUTTER ENZYMS ON THE BASIS OF TOTAL NITROGEN

The fat in butter acts as so great a diluent for the water-soluble constituents that a direct comparison of the enzyms of milk and butter is misleading. The more logical comparison is on the basis of the total nitrogen, since the proteins and enzyms exist in the same colloidal state and ought to be carried into the butter in the same proportions as they exist in the cream. The total protein of the fresh dairy butter was 0.55 per cent, that of the milk from which it was made 3.47 per cent, so the enzymic activities in the butter were multiplied by 6.31. On taking the milk as the standard and its enzymic content as 1, butter was found to contain the following amounts of the various enzyms which were studied:

Galactase	1.100	Catalase	2.000
Oxidase	0.000	Peroxidase	0.008

The galactase content of the butter was approximately equal to that of the milk, the catalase content was double, but the peroxidase content was very much less, and no oxidase activity could be detected.

RELATION OF ENZYMS IN BUTTER TO DETERIORATION DURING STORAGE

As mentioned in the introductory paragraphs of this paper, deterioration in quality of butter during storage has been considered by some investigators to be due to the action of enzyms contained in it. Fat-splitting (lipase) or protein-hydrolyzing ("galactase" or casease) enzyms have been suggested as possible agents in causing deterioration. Our work leads us to conclude that lipases are present in butter in very small amounts, if at all, and that they could not be conceived to be sufficiently active at the low temperature used in butter storage to cause any appreciable change in the quality of the butter. The protein-hydrolyzing enzym we found to be completely inhibited by sodium chlorid in the concentrations which are present in the water contained in all normally salted butters. This fact, together with the known inhibiting effect of low temperatures upon proteolysis by enzyms makes it impossible that the hydrolysis of proteins in the butter by enzyms plays any part in deterioration changes. We conclude, therefore, that enzyms are not to be considered as a factor in the deterioration of butter in cold storage.

SUMMARY

(1) Proteolysis in skim milk was completely inhibited by 1 per cent of chloroform and by 15 per cent of sodium chlorid. Galactase can not act in normal butter because of the high salt content.

(2) In the separation of milk the factors which increase the percentage of casein in the total nitrogen also increased the galactase content. The ripening of cream did not increase the rate of proteolysis.

(3) No oxidase was found in milk or butter.

(4) Only one sample of butter gave any evidence of lipase at the end of four days at 40° C.

(5) The enzym content of butter is very small, because of the high dilution in fat. Expressed on the basis of total nitrogen the butter examined contained as much galactase as fresh whole milk, twice as much catalase, but only one one hundred and sixtieth as much peroxidase.

(6) The cold storage of butter weakens the peroxidases, but has little effect on the catalase and galactase.

(7) Enzyms are present in butter in such small amounts and under such unfavorable conditions for enzym activity during cold storage that they need not be considered as a factor in the deterioration of butter during storage.

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